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MICROBIOLOGICAL QUALITY OF RAW MILK IN BHUTAN

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ABSTRACT: A two-year longitudinal study was undertaken with the objective to establish a bench-mark for microbiological quality of milk in Bhutan. A total of 2191 milk samples were collected from nine functional and relatively more organized farmers' dairy groups located in eight different districts and two government dairy farms. Samples were aseptically collected and subjected to rapid field mastitis tests, followed by laboratory culture, isolation, identification and further characterization. The study revealed that 89% of the samples had udder infection, with prevalence rate of sub-clinical and clinical mastitis at 67% and 20.7%, respectively. This indicates contamination of raw milk with both contagious and environmental pathogens. A variety of pathogenic organisms of 18 different groups comprising 944 isolates were isolated and identified. There was a significant prevalence of anti-microbial resistance for milk borne pathogens. There was also a significant seasonal variation in the microbiological quality of raw milk. Based on this study, there is a need to improve the microbiological quality of milk through adoption of hygienic milk production techniques and improved udder health control programme.

Keywords: Raw milk; microbiological quality; mastitis; pathogens; udder health.

1. INTRODUCTION

The Renewable Natural Resources (RNR) sector aims to achieve self-sufficiency in milk and milk products. The paradigm shift in production system from subsistence production system in the past to commercialization of milk production may compromise the microbiological quality of milk. This shift may have its own detrimental consequences in terms of microbiological quality. Pressures on production system intensification may result in production diseases like mastitis. The emerging concern on human health safety and a rapid growth of dairy industry demand raw milk of good quality, both microbiological and compositional along the whole value chain. Low microbiological content in raw milk prior to processing is essential, as the quality of end products depends on quality of raw milk. In Kerala, as per the PFA standard, the microbiological quality of milk should not exceed 30000 SPC ml^{-1} and coliform bacteria must be absent in 0.1gm of milk (Department of Dairy Development 2010).

However, in Bhutan, there is no information on microbiological quality of raw milk. The faecal samples were collected in the morning from the freshly defecated patch in 30g plastic vials. The fecal sample was analyzed by using three methods of Stoll, sedimentation and floatation, for indicating

different types of worm prevalence and egg count. Thus, this study was undertaken with the objectives to generate information on microbiological quality of raw milk and document important milk borne pathogens in Bhutan, along with antimicrobial susceptibility testing (AST) and influences of other factors on the microbiological quality of milk.

2. Materials and Methods

2.1 Study locations

The study locations are presented in Figure 1. Nine functional farmers' dairy groups from different districts were purposively selected for the study (Table 1). The reasons for their selection were accessibility, existence of functional farmers groups and milk processing units (MPUs). They also represented different agro-ecological zones (AEZs) of the country. However, the over-riding concern in selecting these groups was the public health aspect. These groups produce relatively higher volume of milk and have higher number of consumers for whom there is safety implication, should the milk quality be unacceptable from microbiological aspect. Beside the farmers' groups, the study was also extended to government farms viz. Brown Swiss Farm in Bumthang district and National Jersey Breeding Centre in Samtse district. The study comprised of milk sampling and testing at field and laboratory at National Centre for Animal Health (NCAH).

2.2 Sampling

Sampling was done twice in all locations; once each in summer and winter to take into account the possible seasonal variations. In the farmers' groups or cooperatives, sampling was done at household level (composite sampling) on the first day, followed by individual cow level on the second day, and inspection of cow with sampling on the third day when required. At the organized government dairy farms, sampling was done at individual cow level on day one, at individual udder level on day-two and revisiting the individual cow with sampling when needed on day-three.

Also, at every MPU and government farm, bulk tank milk samples were taken to evaluate the quality of bulk tank milk. Every dairy cow at farmers' level and every udder at farm level were included in the study. In total, 2191 milk samples were collected and tested. About five milliliters (ml) of milk was aseptically collected in sterile bijou bottle with screw-cap as individual sample. Samples were coded, stored in cool boxes and transported to laboratory.

2.3 Field testing

In the field testing, all samples were firstly subjected to California Mastitis Test (CMT), using the standard protocol of Ruegg (2005) and Mellenberger (2001). The individual result obtained was scored as either N, T, 1, 2 or 3, as per the interpretation provided in Table 2. In the second field test, again all samples were subjected to White Side Test (WST), as per the modified protocol of Schalm et al. (1971) i.e. 4% NaOH and milk at 3:1 ratio. The grading and interpretation of WST were same as that for CMT. The rapid tests were further validated (in the field itself at field veterinary laboratories) through somatic cell count (SCC) by breed smear technique. All samples with CMT and

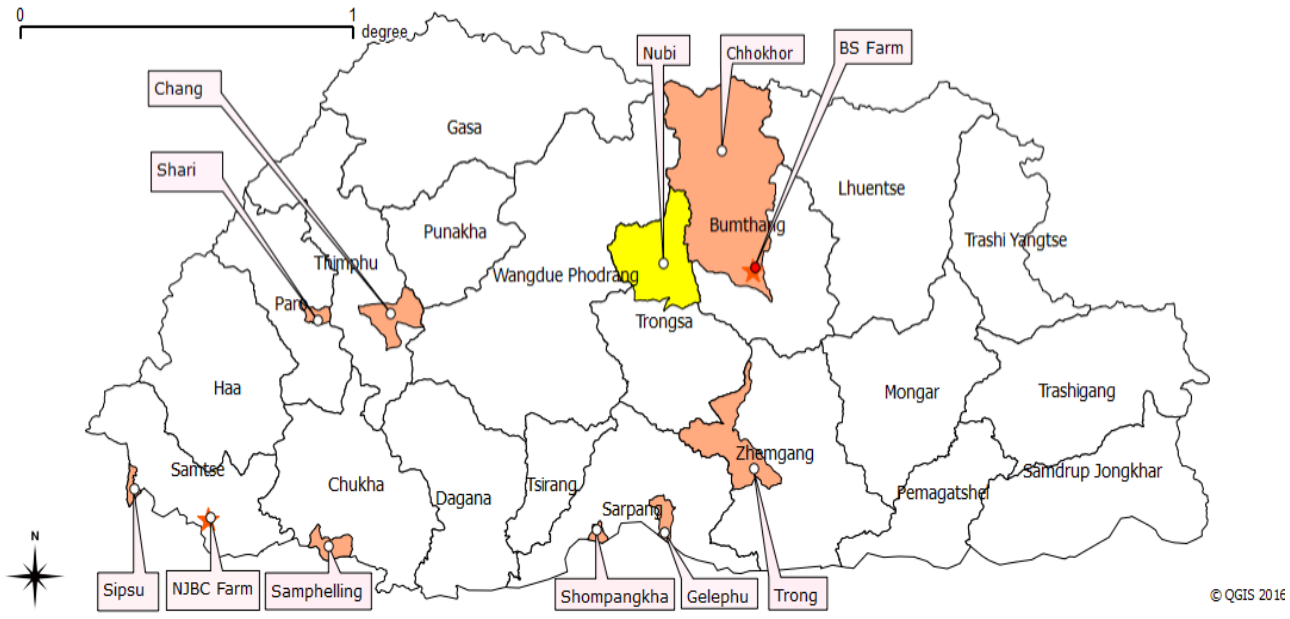

Figure 1: Study locations.

Table 1: Farmers' groups and government farms

Sl No	Farmers groups/Farm	District	Region
Community farm			
1	*Rama Om Tshogpa*	Thimphu	West
2	*Shari Lothuen Om Tshogpa* (SLOT)	Paro	West
3	*Samphelling milk Tshogpa*	Chukha	West
4	*Choling Yargay Daytshen*, Hangay	Samtse	West
5	Gelephu Milk *Detshen*	Sarpang	East-Central
6	*Lothuen Om Detshen* (LOD)	Sarpang	East-Central
7	*Trong-Dangkhar Gonor Chethuen Tshogpa*	Zhemgang	East-Central
8	*Nubi Om Phenden Tshogpa*	Trongsa	East-Central
9	*Chokhor Gonor Gongphel Tshogpa*	Bumthang	East-Central
Government farm			
10	National Jersey Breeding Centre (NJBC)	Samtse	West
11	Brown Swiss Farm (BSF)	Bumthang	East-Central

Table 2: Interpretation of CMT scores.

CMT Score	Somatic Cell Range	Interpretation
N (Negative)	0 – 200,000	Healthy Quarter
T (Trace)	200,000 – 400,000	Subclinical Mastitis
1	400,000 – 1,200,000	Subclinical Mastitis
2	1,200,000 – 5,000,000	Serious Mastitis Infection
3	Over 5,000,000	Serious Mastitis Infection

WST scores of 1, 2 and 3 were aseptically transferred into HiCulture™ Transport Swabs w/Amies Medium w/Charcoal MS651 for culture, isolation and identification of bacterial pathogens at NCAH. A few negative (negative to CMT and WST) samples were also included for culture as controls to validate the field rapid tests. The FAO's guidelines like CAC/GL 21-1997 and CAC/GL 63-2007 were also taken into consideration while undertaking this study (FAO 1997 and 2007)[4,5]. CMT scores are directly related to average somatic cell counts. Any reaction of T (trace) or higher indicates that the quarter has subclinical mastitis.

2.4 Laboratory testing

The samples were finally cultured on Sheep Blood Agar (SBA) and MacConkey Agar at National Veterinary Laboratory of NCAH. The isolates were then subjected to various bio-chemical tests (stage 1, 2 and 3). Each isolate was sub-cultured to purify, multiply and identify the pathogenic organism. AST was performed for major pathogens isolated by Disk Diffusion Test (Kirby-Bauer method). Mueller Hinton Agar plate and antibiotic impregnated disc, containing antibiotics as per standards of the Clinical and Laboratory Standards Institute (CLSI) were used. A panel of seven antibiotics commonly used in the field were included viz. Ampicillin, Amoxicillin, Tetracycline (broad-spectrum antibiotics), Penicillin G, Erythromycin (G+ narrow-spectrum antibiotics), Gentamycin and Streptomycin (G- narrow-spectrum antibiotics).

Result of AST was interpreted on the diameter of zone of inhibition to nearest mm based on "Zone size interpretation chart" (modified from National Committee for Clinical Laboratory Standards (NCCLS) M2 A4: 1990), which is a performance standard for antimicrobial disk susceptibility tests. The results were classified into R (resistant), I (intermediate) and S (susceptible). Moderately susceptible class, as per above zone size interpretation chart, was clubbed with intermediate for convenience.

2.5. Statistical analysis

One sample t-test was conducted to test difference in pathogen population. The dataset was analysed with SPSS version 20 and Microsoft Excel.

3. RESULTS

3.1 Important milk borne pathogens/bacterial

From the standard bacteriological cultures, 994 major isolates belonging to 18 broad groups were obtained (Figure 2). The list included both contagious and environmental pathogens. The top ten commonest milk borne pathogens are presented in Figure 3.

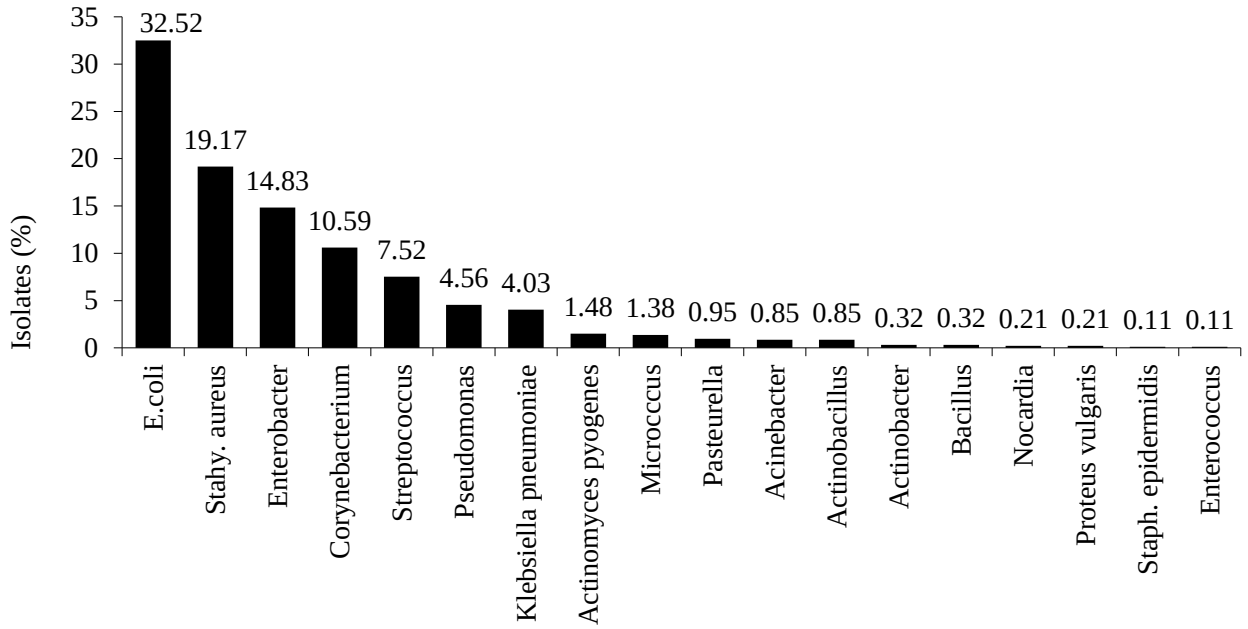

Figure 2: Total milk borne pathogens.

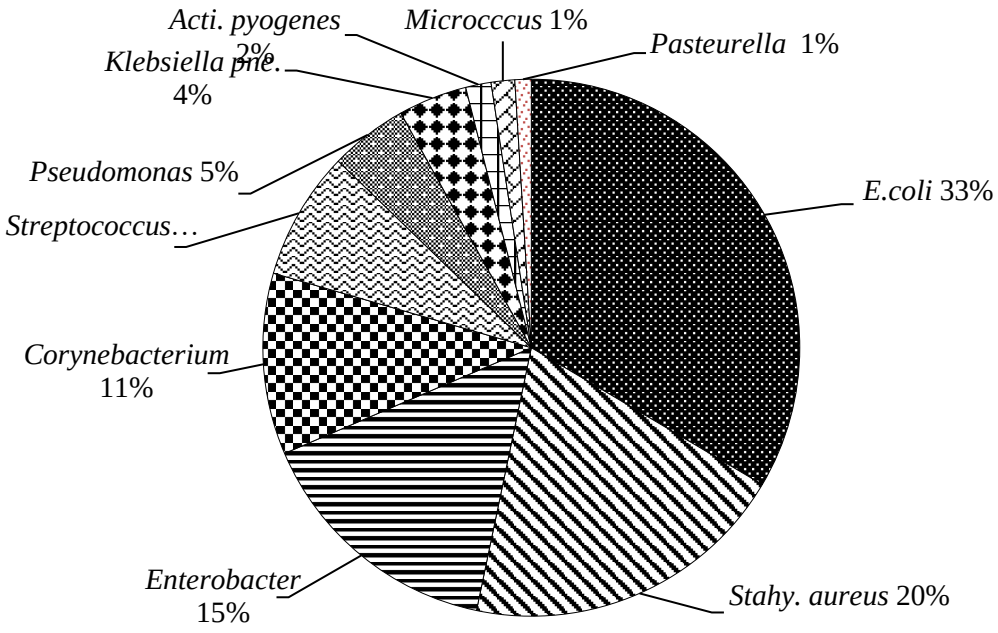

Figure 3: Ten commonest milk borne pathogens.

3.2 Antimicrobial Resistance (AMR) of isolates

There was a significant difference (prevalence of anti-microbial resistance) between sample mean and hypothesized mean, indicating presence of anti-microbial resistance in milk borne pathogens. The AST profiles are given in Table 3, 4 and Figure 4.

3.1 Seasonal variation and mastitis prevalence

There was a significant seasonal variation of mastitis with a higher prevalence in winter (Figure 5). Compared with serious mastitis, the prevalence of sub-clinical mastitis was significantly higher in both summer and winter. This study revealed a very high bovine mastitis prevalence rate of 20.7% for clinical mastitis and 67% for sub-clinical mastitis. Among the results obtained, this is one of the most important baseline information generated.

4. DISCUSSION

The most common mastitis causing organism was found to be *Escherichia coli* (*E. coli*), followed by *Staphylococcus aureus* and Streptococcus. This is similar to the findings of Bradley (2001), Miltenburg (1996), Schukken (1989), Burvenich (2003), and Petrovski (2006). Presence of both

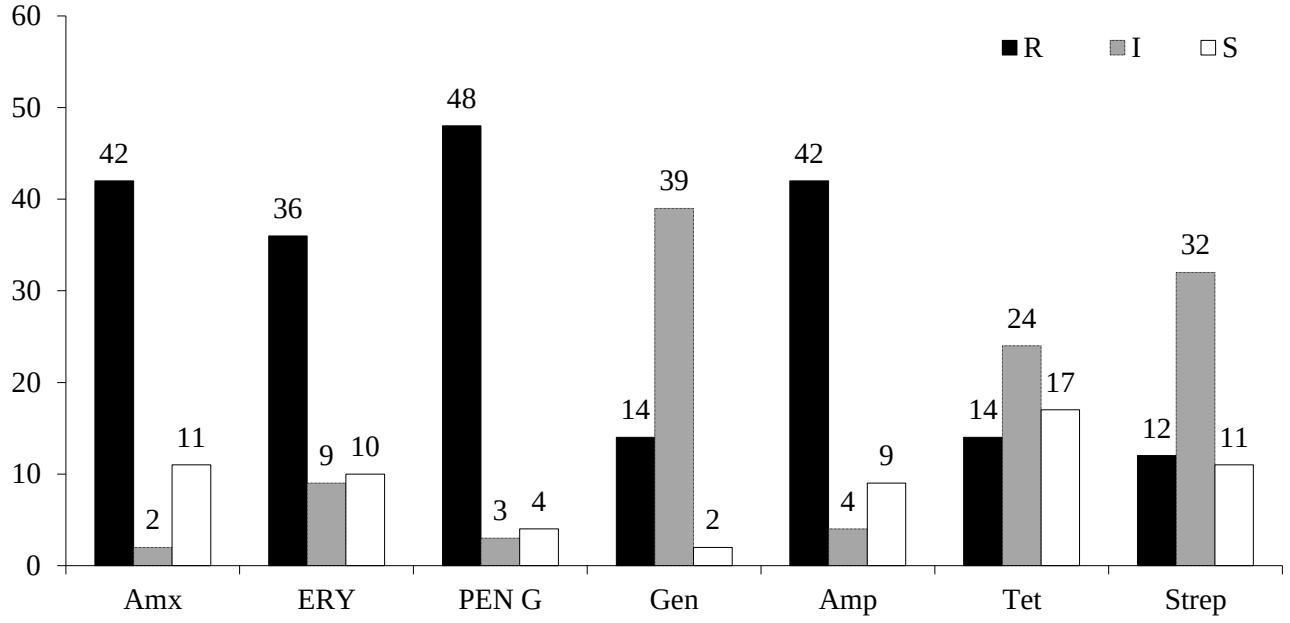

Figure 4: AST profile.

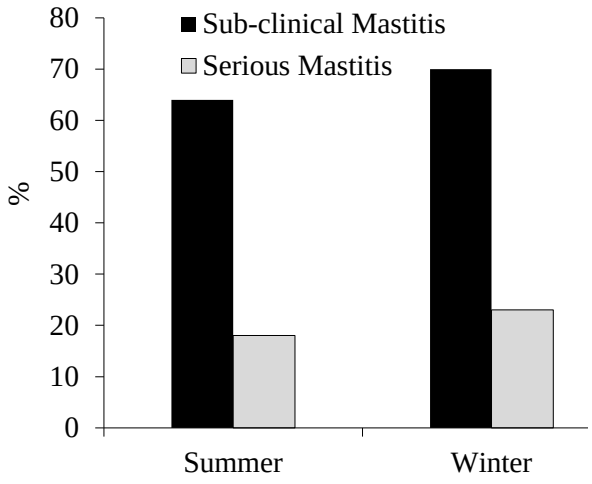

Figure 5: Seasonal mastitis prevalence.

contagious and environmental pathogens indicates pathogenic organisms having entrenched in our dairy herds. The environmental factors related to poor management, particularly the housing part contribute to high microbial load.

Since there is no strict legislation or guideline for anti-microbial usage for mastitis treatment and control, some levels of antibiotic resistance are expected in such a scenario. Resistance proportion ranging from 23% for penicillin G to 6% for Streptomycin is comparable with the findings of Saini et al. (2012). The high proportion of antibiotic resistance for penicillin G was also found by Botrel et.al (2010) who estimated the distribution of pathogens, as well as their antimicrobial resistance pattern, in cows affected by clinical or subclinical mastitis in the Rhône-Alpes region of France. Thus, this study stipulates the need for strategies to

Table 3: AST by most sensitive and most resistant.

Most sensitive	Most resistant
Tetracycline 27%, Streptomycin 17%, Amoxicillin 17%	Pen G 23% Ampicillin 20% Amoxicillin 20%

Table 4: AST profile (S=Susceptible, I=Intermediate, R=Resistant).

	%		
Isolate	S	I	R
Penicillin G	6.00	3.00	23.0
Gentamycin	3.00	34.0	7.00
Tetracycline	27.0	21.0	7.00
Streptomycin	17.0	28.0	6.00
Amoxicillin	17.0	2.00	20.0
Erythromycin	16.0	8.00	17.0
Ampicillin	14.0	4.00	20.0

encourage prudent use of antimicrobials as put forward by Oliver et al. (2012). A study at Michigan State University by Erskine (2002) showed that there is no indication of increased resistance of mastitis isolates to antibacterial that are commonly used in dairy cattle. This was made possible through prudent use of antimicrobials aided by strict legislation on the use of antimicrobials.

Bacterial antimicrobial resistance in both medical and agricultural fields has become a serious concern worldwide. Research has linked the use of antibiotics in agriculture to the emergence of

antibiotic-resistant food borne pathogens (McDermott et al. 2002).

Although the seasonal effect on prevalence of mastitis was unexpected, we found that the prevalence was significantly higher in winter. This is in agreement with the finding of highest clinical mastitis incidence rate in December to January (Olde Riekerink et al. 2007). The higher winter prevalence could be attributed to poor personal and equipment hygiene under the freezing cold in winter.

Even though, a high prevalence rate was detected, it is comparable to existing prevalence rates in Asia (Sharma et al. 2012) who showed an increasing trend of bovine mastitis with a prevalence of >70% for India, >60% for Pakistan and Nepal, >50% for Bangladesh, South Korea and China. However, given the free veterinary services provided by the government in Bhutan, it can be brought down significantly with an improved udder management and addressing the other risk factors of bovine mastitis.

5. CONCLUSION

This study revealed a large number of bacterial pathogens responsible for bovine mastitis. The definite prevalence of anti-microbial resistance too was proven. A high prevalence rate of bovine mastitis with higher prevalence in winter season was established. To address the issue of poor microbiological quality of milk, a bovine udder health control program may be instituted in near future. Although, this study provided lot of information on microbiological quality of raw milk, there is a need to address the gaps in this study and establish more valid benchmarks. Some of the areas to focus in future studies are correlation with other host factors viz., breed, lactation number, lactation stage, age, parity, length of dry period, milking interval and environmental factors like housing, nutrition, hygiene, milking techniques etc. Besides these, improvement in the overall study design, including laboratory protocols, is crucial.

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REFERENCES

Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A and Duchateau L (2003). Severity of *E. coli* mastitis is mainly determined by cow factors. Veterinary Research, 34 (5): 521-64.

Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY and Calavas D (2010). Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. Foodborne Pathogens and Disease, 7 (5): 479-87.

Bradley AJ and Green MJ (2001). Aetiology of clinical mastitis in six Somerset dairy herds. Veterinary Record, 148 (22): 683-6.

Department of Dairy Development (2010). Government of Kerala. Available at: http://www.dairy.kerala.gov.in/index.php/quality-control/pfa-standards-of-milk. (Accessed on 19th March 2018)

Erskine RJ, Walker RD, Bolin CA, Bartlett PC and White DG (2002). Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. Journal of Dairy Science, 85 (5): 1111-8.

FAO (2007). Principles and guidelines for theconduct of microbiological risk management (MRM) CAC/GL 63-2007. Available at: file:///C:/Users/user/Downloads/CXG_063e.pdf , (Accessed on December 3, 2016).

McDermott PF, Zhao S, Wagner DD, Simjee S, Walker RD and White DG (2002). The food safety perspective of antibiotic resistance. Animal Biotechnology, 13 (1): 71-84.

Mellenberger R (2001). California Mastitis Test (CMT)- An Invaluable Tool for Managing Mastitis. Dept. of Animal Sciences, Michigan State University. Available at: http://immucell.com/wp-content/uploads/An-Invaluable-Tool.pdf. (Accessed on 20 February 2018)

Miltenburg JD, de Lange D, Crauwels AP, Bongers JH, Tielen MJ, Schukken YH and Elbers AR (1996). Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands. Veterinary Record, 139 (9): 204-7.

Olde Riekerink RG, Barkema HW and Stryhn H (2007). The effect of season on somatic cell count and the incidence of clinical mastitis, 90 (4): 1704-15.

Oliver SP and Murinda SE (2012). Antimicrobial resistance of mastitis pathogens. Veterinary Clinics of North America, Food Animal Practice, 28 (2):165-85.

Petrovski KR, Williamson NB, Lopez-Villalobos N, Parkinson TJ, and Tucker IG (2006). Culture results from milk samples submitted to veterinary diagnostic laboratories from August 2003 to December 2006 in New Zealand. New

Zealand Veterinary Journal 2011 Nov;59(6):317-22.

Ruegg PL (2005). California Mastitis Test (CMT) Fact Sheet 1 & 2. Available at: http://milkquality.wisc.edu/wp-content/uploads/2011/09/california-mastitis-test-fact-sheet.pdf, http://milkquality.wisc.edu/wp-content/uploads/2011/09/california-mastitis-test-fact-sheet-2.pdf. (Accessed on 23 February 2018).

Saini V, McClure JT, Léger D, Keefe GP, Scholl DT, Morck DW, and Barkema HW (2012). Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. Journal of Dairy Science, 95 (8): 4319-32.

Schukken YH, Kremer WD and Lohuis JA (1989). Escherichia coli mastitis in cattle. I. Clinical diagnosis and epidemiological aspects. Tijdschr Diergeneeskd, 114 (15-16): 829-38.

Sharma N, Rho GJ, Ho Hong Y, Kang TY, Lee HK, Hur TY and Jeong DK (2012). Bovine Mastitis: An Asian Perspective. Asian Journal of Animal and Veterinary Advances, 7: 454-476.